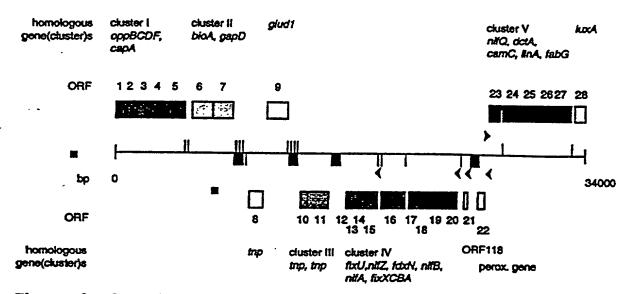


15,783 (vertical line in the middle of each sequence). The readings cover a part of the minus strand of pXB296. (♥) Extremely low signals produced with dye terminators; (♣) the sequence GCA, which is Figure 1. Comparison of sequences from pXB296 created by different cycle sequencing methods. The graphic outputs (program XGAP) of four electropherograms (traces) with the corresponding sequences generated by automatic base calling are shown around the pXB296 sequence position compressed in dye primer scans; (•) automatic base-calling inaccuracies.



**Figure 2.** Organization of the predicted ORFs in pXB296 from *Rhizobium* sp. NGR234. Significant stem-loops ()/stem-loop clusters ( $\blacksquare$ ), which might function as p-independent transcription terminators, are represented. Sequence motifs (open arrowhead), similar to  $\sigma^{54}$ -dependent promoter consensus sequences (TGCCACG-N<sub>4</sub>-TTGC) + nifA upstream activator sequences (TGT-N<sub>10</sub>-ACA), are located at the following positions on the cosmid: 19,107–19,120 + 19,195–19,210; 24,787–24,800 + 24,878–24,893; 25,508–25,521 + 25,567–25,582; 26,815–26,828 + 26,941–26,956 (all minus strand); 27,074–27,131 (several possibilities) + 26,969–26,984 (plus strand).

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NGR234a.gcg Thu Jun 27 11:21:16 19961

Complete sequence of pNGR234a:

# Figure 3

Length: 536165 bp

1 gatotogatt ggcagaaccg gcaccgcgcg ccagctaact acgccataga 51 gegegageag gaagegegtg agtacgttge tgttggegge geegeegege 101 gegeggateg cetegegege etteaceata tgageegegt egaeggaate 151 accgatcatc ttgagegega agtaagactt caegettgeg etcatatega 201 agggcccgtc atgcaccage ggccagccgc cgtgcgcgcc ttgagtgcgg 251 cgcagataat ttccgatttt ggcttcgagc acgacgtcaa tgggctcggc 301 caggtaatga cgtagcagga tgtattcgga agggatggtg gaatccgctt caagetegaa egeceaatgg eegteggeat gaegataage gageagegee 351 401 tcagtggctg aggcgatgct catttccagc gcggcgggat cgatggcagt 451 gcgatttccg gaatgcttat tcactgactt cctgcgtgtt cggcagcgtg 501 caaaaccgtc tggatttatc aatcaagtgt ttgtctctga aagagccaga 551 toggoggcac ggttaccoga cogcacogat coctogatgg ttgccggcaa 601 accygttycy gtccagtcyc cagcaaggaa caggttttty caaccygtca 651 cgggccctgg acgtagcgcg ttctgctccg gggtggcctg gaatgtggcc 701 cggcgctcac acacgatctg ccacggcggc agetegectg agateceaec 751 ggcctcgcac acgtcgcgcc agatcactcg cacgacctcc tcacgcggaa 801 tgtcaagcag geggtegeea ttgctaatgg tgactgaaag eegetgegga 851 taggogaaca gocactocac gagocogoog accaegocca ggatoggato 901 cgcaccgact ggcggatcga agcgaaaatg ggcattaacg acggcgcgga 951 attcggtcgg ggttttcagg ccgggcagga gcgtcgcggc cgcccgtggc 1001 ggcacggcga ggatcacggc gtcgtcgggg ccgaccgcta tcttgtcgtc 1051 accgaaatca agttegetga tgatetegge egatttggea agettgegea 1101 gtttatggct gaggcggacg gtggcgcccc gcctctccag gagcttgacc 1151 gccggctcga ccagaacggc gctcagtccg tcgcgcgcga ctagcggacg 1201 gcaagecteg eegecegega geagegttte eegtaegatg geaceggeaa 1251 gccctgccga gccctccggc ggatcgcaat tcagggcggc gagcagcagc 1301 ggccgcacca gacgccggta cagcgtacca ttgcagggta tagtgtttcc 1351 gaccagttca teegegeegg cecataggat tggegegage tteaggtagt 1401 cccacageet ggtgtegggg aegegaegeg cettgtggaa cacecaggte 1451 ggaageetge egeegeegag ategacetge cagegetgta eggtggagat 1501 gtcgacgaag ggaaacttgg cactcgtcgg accgacaaga ccggattccg 1551 ttccgatcgc acgggcgtag ttgcgcacat attggttgcc cgatagaacc 1601 agatggttgc cgttgtcgat ggtgagattg gttgcggaat cgaaaaagga 1651 geggeatega eegeeggeet gttgegtege tteatagaea tgeaceggea 1701 aacctgcatt gctcaactgc acggccgcgg aaaggccgga gatcccggcg 1751 ccgataatgt gaacattttt tggcatcaca acatcaaggc gtaacggaag 1801 agaatggcgc gctttgtgag attggttaca cgcaccggtt cccgggggagc cgcaaaaccc cttatcagca gcaggtccaa gatcgcgcgg taacatttgg 1851 1901 acatgatggt gggggcgcgt actattctgc gcgggttgca attcatgatc 1951 togtoggatt oggoaaaatg catcatogog ogttoggoca goggtgogoa 2001 cgccttgggc agcgccttgt cggcgatgac tttatgtggg tcgctgctgg 2051 tgataccggc gtgatcgagg ctttcgcgtg gtatatagag ccgacccagc 2101 ccagcatect egtetatgte gegeaggatg ttggtgagtt geagegeacg 2151 accgaggtga tgcgcaagtg caatgccatc ctcctcgctc aagccgaaca 2201 cacgcaccga catecttece acggcgctgg cgacacggtc gcaatagaga 2251 tccagggttg ccattttggg cgcgcggatg tcttgtagta cgtccatttc 2301 catgoogtog acaacggota ggaaatcoto togottoago cogaaggtog 2351 ttaccgagge gagatagtee tttageegeg geggeggtae acattggtag 2401 agegegteaa tgtgategeg eeattgetga agtgeageaa ggeggtgete 2451 gcgcggttcg tcggagtcgg cgatgtcgtc gacctggcgg cagaagctgt 2501 agatotggaa tatogooteg ogotgoaceg gegggagggt acgcattece 2551 agatagaacg agctgccaag ggccgtagag cggtggttcg cgtgggccgc 2601 cgcctccgcc ctcatacgtc gacagtcgtc ttgaatcgcg taccgcggcc gaacgcacgg cgcgcgctt cgtttgccat tgcgccgacg ccatggagca 2651 2701 grageteaag eggegacaga tgcacgeget ceettagagg ategegeate ttcagcatcg acacgatctt atcggcaaaa gacagtatga cggagacctc gaaccggagg cgaaagteet teacctgega caccageget cegecceegt 2801 2851 tgagcagcac cteggeette getgegagag agegeaggea ttgtagcate tgcaacgagg attttgcggc gcccagctct tcgatggagg cgccgctggc 2901 tgaaagcgca tcgcgcggaa tatagacgcg attgagatta aggaaatcct 2951 3001 tgccgcaatc ctgcaggtgg ttacagacct gcagtcccgc gcacaacaca 3051 tocgatgoog gooacgtogo ggoactttog cogtggacgt cgagcacaaa toggoogaec ggcatogoog agtatoggca ataatogato acctogtoco 3101 3151 agttotogta acgotgotta gtoacgtoca ttoggaaggo ggtgagcaga 3201 togagtgcat gacgoggogg catgoogogt oggtcaagog cagtgogtag 3251 gtgtacaget tetgetegeg tttegecett geegageage teggetteca ggaggtcgag gtacagaagc ttctcgtctg gcgcgagcgt cgcgtgatcg 3301 gcaatgtcat cggccatgcg gacgaatcgg tagaaggcaa gaatcagcgc 3351 3401 toggtgccgc ggatggataa tocacgacgc aacggggaaa tttttctcgc ggtgcattgc gtccgacctg gggccgctgc cgctcgtcat ttagcaccat 3451 3501 cgtcggtttt gcaggcgttg gatgctgctg aaatgcagtc gggcccccga

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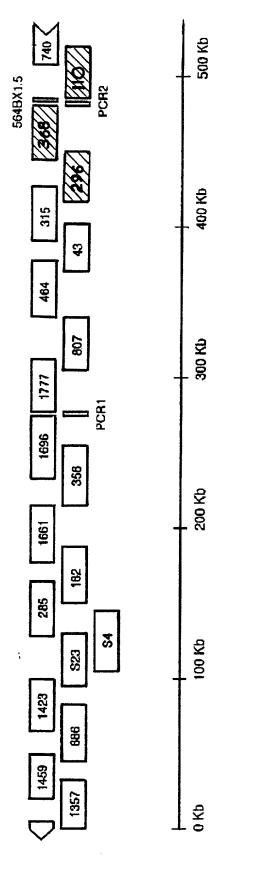
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536051 536101 536151	taggattctt	cgcacgcggc gcgcccagta		caagaaccat	cageggeaca

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564BX1.5 and PCR2: a subcloned 1.5-Kb DNA-fragment derived from cosmid pXB564 and a PCR product covering the gap between two Figure 4: Map of the 20 sequenced cosmids covering the 536-Kb symbiotic plasmid of *Rhizoblum* sp. NGR234. PCH1: PCR product covering the gap between two cosmids from position 276,448 to 277,944. The cosmid names consist of the prefix pXB and numbers that are given in the graphic. cosmids from pos. 480,607 to 483,991.

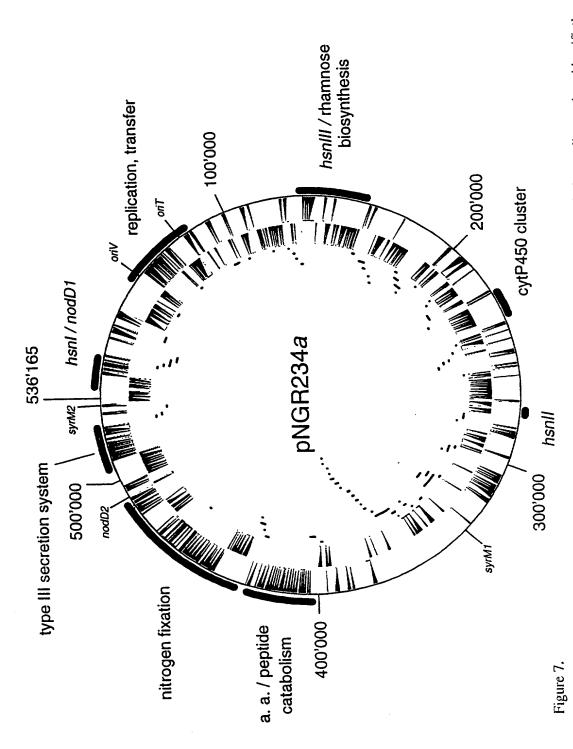
Grey coloured areas cover the region from pos. 417,796 to 517,279 mentioned in the text.

Multiple alignments of the nucleotide sequence of the replication origins of: the

Ri plasmid of Agrobacterium rhizogenes (pRiA4b), the symbiotic replicon of NGR234 leguminosarum bv. leguminosarum (pRL8JI). Gaps introduced to give the best sequence (pNGR234a), the Ti plasmid of A. tumefaciens BS63 (pTiBS63) and pRL8JI of R. alignments are marked by hyphens.

	-	10	20	30	
pNGR234a	CAGGATG	TCGCGACAG	CGACGTA	ATGTCGCGACGT ATAATTGCGCCCTGGA	<b>Y</b>
pTiC58	CAAGGCG	TCGCGTCAG	CGACGTA	GCGTCGCGTCAGCGTATAATTGCGCCCTTGG	D C
RSF1010	CCAGTTT	CTCGAAGAG	AAACCGG	CCAGTTTCTCGAAGAGAACCGGTAAGTGCGCCTCCC	သ
pTF1	GGGTAAT	CTCGAAGAG	BATTACTC	AATCTCGAAGATTACTCTAAGTGCGCCTTGC	၁ ၅

ferrooxidans. Major conserved nucleotide residues are boxed. Known "nick" sites corresponding to the nucleotide positions where the specific plasmid strand is cleaved are marked (z). Sequence features in the trailing portion of the oriT sites include inverted repeats Multiple DNA sequence alignments of the regions containing the origin of transfer of: the symbiotic plasmid of NGR234; pTiC58, the Ti plasmid of A. tumefaciens C58; RSF1010, a mobilisable plasmid of E. coli, and; pTF1, a mobilizable plasmid of Thiobacillus which are marked by horizontal arrows. Figure 6.



Circular representation of the symbiotic plasmid of NGR234. Outer and inner concentric circles: coding regions identified on the plus and minus strands respectively. ORFs which belong to insertion-like elements are not visible. Thin concentric black lines represent mosaic sequences as well as complete or partial IS like repeats. Major gene clusters are highlighted as thick black concentric lines.